

FORM PTO-1390
(Rev. 9-2001)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

**TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371**

029650-111

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5)

Unassigned **10/018930**INTERNATIONAL APPLICATION NO.
PCT/JP00/04182INTERNATIONAL FILING DATE
26 June 2000PRIORITY DATE CLAIMED
25 June 1999TITLE OF INVENTION
LIPOSOME

APPLICANT(S) FOR DO/EO/US

Masayo KONDO; Takashi HARIGAI; Junji KIMURA and Masashi ISOZAKI

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. ☐ is attached hereto (required only if not communicated by the International Bureau).
 - b. ☒ has been communicated by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. ☒ is attached hereto.
 - b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
 - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
 - b. ☐ have been communicated by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A **FIRST** preliminary amendment.
14. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☒ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☒ Other items or information: Copy of front page of WO 01/00174 A1; International Search Report

**21839**

U.S. APPLICATION NO. (If known, see 37 CFR 1.51)

Unassigned

10/018930

INTERNATIONAL APPLICATION NO.

PCT/JP00/04182

ATTORNEY'S DOCKET NUMBER

029650-111

21. ☒ The following fees are submitted:

CALCULATIONS

PTO USE ONLY

Basic National Fee (37 CFR 1.492(a)(1)-(5)):

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$1,040.00 (960)

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$890.00 (970)

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$740.00 (958)

International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$710.00 (956)

International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) \$100.00 (962)

ENTER APPROPRIATE BASIC FEE AMOUNT =

\$ 890.00

Surcharge of \$130.00 (154) for furnishing the oath or declaration later than months from the earliest claimed priority date (37 CFR 1.492(e)).

20 ☐ 30 ☐

\$ 0

Claims	Number Filed	Number Extra	Rate
Total Claims	19 -20 =	0	X\$18.00 (966)
Independent Claims	4 -3 =	1	X\$84.00 (964)
Multiple dependent claim(s) (if applicable)			+ \$280.00 (968)

\$ 0

\$ 84.00

\$ 0

TOTAL OF ABOVE CALCULATIONS =

\$ 974.00

Reduction for 1/2 for filing by small entity, if applicable (see below).

+

\$ 0

SUBTOTAL =

\$ 974.00

Processing fee of \$130.00 (156) for furnishing the English translation later than months from the earliest claimed priority date (37 CFR 1.492(f)).

20 ☐ 30 ☐

+

\$ 0

TOTAL NATIONAL FEE =

\$ 974.00

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 (581) per property

+

\$ 40.00

TOTAL FEES ENCLOSED =

\$ 1,014.00

Amount to be refunded:

\$

charged:

\$

a. ☐ Small entity status is hereby claimed.b. ☒ A check in the amount of \$ 1,014.00 to cover the above fees is enclosed.c. ☐ Please charge my Deposit Account No. 02-4800 in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.d. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-4800. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Platon N. Mandros
BURNS, DOANE, SWECKER & MATHIS, L.L.P.
P.O. Box 1404
Alexandria, Virginia 22313-1404
(703) 836-6620

William C. Rowland RN 30889, Esq.
SIGNATURE

Platon N. Mandros

NAME

22,124

REGISTRATION NUMBER

December 26, 2001
DATE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of)
)
Masayo KONDO et al.) Group Art Unit: Unassigned
)
Application No.: Unassigned) Examiner: Unassigned
)
Filed: December 26, 2001)
)
For: LIPOSOME)

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

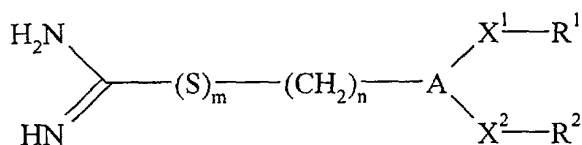
Prior to examination of the above-captioned patent application, kindly enter the following amendment.

IN THE CLAIMS:

Kindly replace Claims 3-16 as follows.

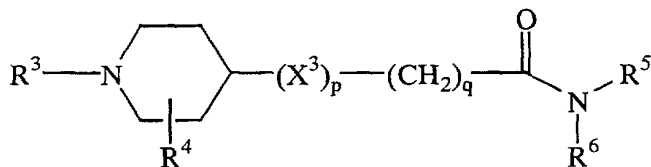
3.(Amended) The liposome according to claim 1, wherein a molar ratio of said acidic compound is 0.5 to 30 mol% of total liposome membrane constituents.

4. (Amended) The liposome according to claim 1, wherein said basic compound is represented by any one of the following Formulae 1 to 4:



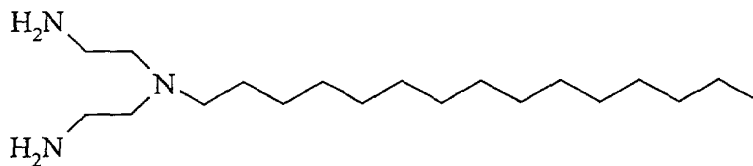
Formula 1

(in Formula 1, A represents an aromatic ring. R^1 and R^2 represent an alkyl group or alkenyl group having 10 to 25 carbon atoms, where R^1 and R^2 may be the same or different. X^1 and X^2 represent -O-, -S-, -COO-, -OCO-, -CONH- or NHCO-, where X^1 and X^2 may be the same or different. m is 0 or 1, and n is 0 or an integer of 1 to 6.)

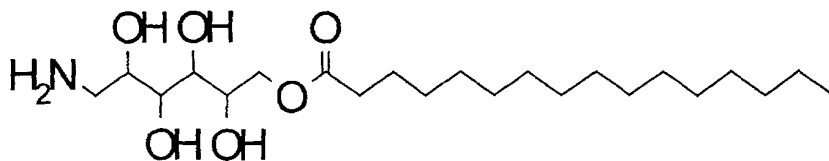


Formula 2

(in Formula 2, R^3 represents hydrogen or an alkyl group or alkenyl group having 1 to 8 carbon atoms. R^5 and R^6 represent hydrogen, or an alkyl group or alkenyl group having 1 to 25 carbon atoms, (except for the case where both R^5 and R^6 are hydrogen atoms), where R^5 and R^6 may be the same or different. X^3 represents -O- or -S-. p is 0 or 1, and q is 0 or an integer of 1 to 10).



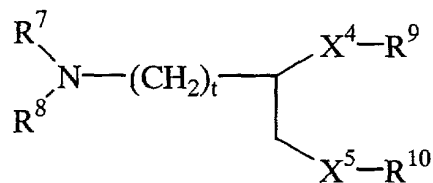
Formula 3



Formula 4

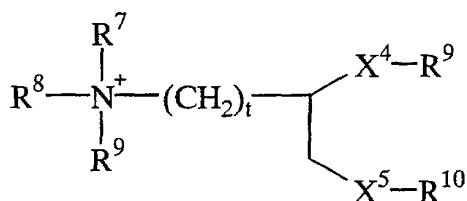
5.(Amended) The liposome according to claim 1, wherein said basic compound is a basic compound having a quaternary amine or a tertiary amine.

6.(Amended) The liposome according to claim 1, wherein said basic compound is selected from the group consisting of the following Formulae 5 and 6.



Formula 5

(in Formula 5, R⁷ and R⁸ represent an alkyl group or alkenyl group having 1 to 8 carbon atoms, where R⁷ and R⁸ may be the same or different. X⁴ and X⁵ represent -O- or -OCO-, where X⁴ and X⁵ may be the same or different. R⁹ and R¹⁰ represent an alkyl group or alkenyl group having 10 to 20 carbon atoms, where R⁹ and R¹⁰ may be the same or different. t is an integer of 1 to 6.)



Formula 6

(in Formula 6, R⁷, R⁸ and R⁹ represent an alkyl group or alkenyl group having 1 to 8 carbon atoms, where R⁷, R⁸ and R⁹ may be the same or different. X⁴ and X⁵ represent -O- or -OCO-, where X⁴ and X⁵ may be the same or different. R⁹ and R¹⁰ represent an alkyl

group or alkenyl group having 10 to 20 carbon atoms, where R^9 and R^{10} may be the same or different. t is an integer of 1 to 6.).

7.(Amended) The liposome according to claim 1, wherein said phosphoric acid monoester derivative is selected from predonisolone phosphate, riboflavin phosphate, and phosphatidic acid.

8.(Amended) The liposome according to claim 1, wherein said compound having a carboxyl group or its salt, is a fatty acid.

9.(Amended) The liposome according to claim 1, wherein said fatty acid is oleic acid, stearic acid, palmitic acid, or myristic acid.

10.(Amended) The liposome according to claim 1, wherein the liposome membrane constituent other than [1] and [2], is phospholipid or its derivative, and/or sterol or its derivative.

11.(Amended) The liposome according to claim 1, wherein said drug intended for the therapy and/or diagnosis is an anti-cancer agent, an antibiotic, an enzyme agent, an enzyme inhibitor, an antioxidant, a lipid uptake inhibitor, a hormone agent, an anti-inflammatory agent, a steroid agent, a vasodilator, an angiotensin converting enzyme inhibitor, an angiotensin receptor antagonist, a growth/migration inhibitor for smooth

muscle cells, a platelet aggregation inhibitor, an anticoagulant, a chemical mediator releasing inhibitor, a vascular endothelial cell growth or suppressing agent, an aldose reductase inhibitor, a mesangium cell growth inhibitor, a lipoxxygenase inhibitor, an immunosuppressor, an immunoactivator, an antiviral agent, a Maillard reaction inhibitor, an amyloidosis inhibitor, an NOS inhibitor, an AGEs inhibitor, or a radical scavenger.

12.(Amended) The liposome according to claim 1, wherein said drug intended for the therapy and/or diagnosis is a nucleic acid, a polynucleotide, a gene and its analogue.

13.(Amended)The liposome according to claim 1, wherein said drug intended for the therapy and/or diagnosis is glycosaminoglycan and its derivative.

14.(Amended) The liposome according to claim 1, wherein said drug intended for the therapy and/or diagnosis is oligo- and/or polysaccharide, and derivative thereof.

15.(Amended)The liposome according to claim 1, wherein said drug intended for the therapy and/or diagnosis is protein or peptide.

16.(Amended) The liposome according to claim 1, wherein said drug intended for the therapy and/or diagnosis is an intracorporeal diagnostic drug such as an X-ray contrasting medium, a radiolabeled nuclear medicinal diagnostic drug, or a nuclear magnetic resonance diagnostic drug for diagnosis.

REMARKS

No new matter has been introduced.

Claims 3-16 have been amended to avoid multiple dependency.

Early and favorable consideration with respect to this application is respectfully requested.

These changes have been made in accordance with 37 C.F.R. § 1.121 as amended on November 7, 2000. A marked-up version of Claims 3-16 indicating the changes is enclosed with this Preliminary Amendment.

Should any questions arise in connection with this application, the undersigned respectfully requests that he be contacted at the number indicated below.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By: William C. Lawrence, RN 30888, for
Platon N. Mandros
Registration No. 22,124

P.O. Box 1404
Alexandria, Virginia 22313-1404
(703) 836-6620

Date: December 26, 2001

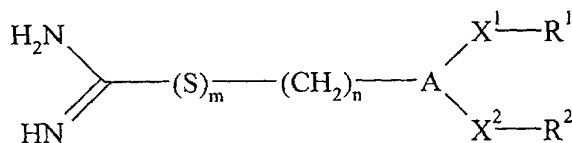
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Attachment to Preliminary Amendment dated December 26, 2001

Marked-up Claims

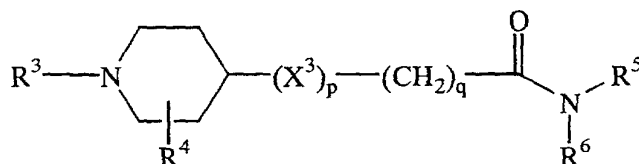
3.(Amended) The liposome according to claim 1[or 2], wherein a molar ratio of said acidic compound is 0.5 to 30 mol% of total liposome membrane constituents.

4.(Amended)The liposome according to [any one of claims 1 to 3] claim 1, wherein said basic compound is represented by any one of the following Formulae 1 to 4:



Formula 1

(in Formula 1, A represents an aromatic ring. R^1 and R^2 represent an alkyl group or alkenyl group having 10 to 25 carbon atoms, where R^1 and R^2 may be the same or different. X^1 and X^2 represent -O-, -S-, -COO-, -OCO-, -CONH- or NHCO-, where X^1 and X^2 may be the same or different. m is 0 or 1, and n is 0 or an integer of 1 to 6.)

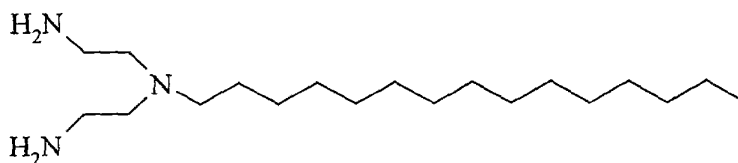


Formula 2

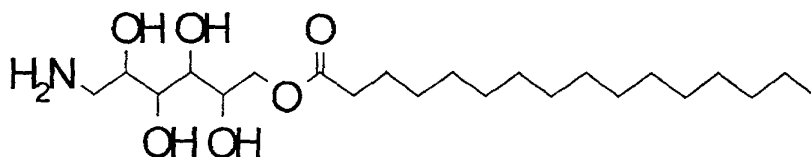
Attachment to Preliminary Amendment dated December 26, 2001

Marked-up Claims

(in Formula 2, R³ represents hydrogen or an alkyl group or alkenyl group having 1 to 8 carbon atoms. R⁵ and R⁶ represent hydrogen, or an alkyl group or alkenyl group having 1 to 25 carbon atoms, (except for the case where both R⁵ and R⁶ are hydrogen atoms), where R⁵ and R⁶ may be the same or different. X³ represents -O- or -S-. p is 0 or 1, and q is 0 or an integer of 1 to 10).



Formula 3



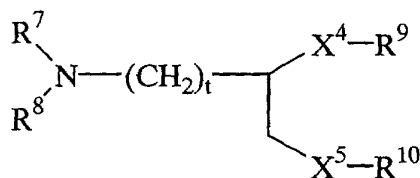
Formula 4

5.(Amended) The liposome according to [any one of claims 1 to 4] claim 1, wherein said basic compound is a basic compound having a quaternary amine or a tertiary amine.

Attachment to Preliminary Amendment dated December 26, 2001

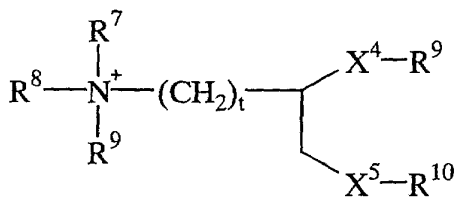
Marked-up Claims

6.(Amended) The liposome according to [any one of claims 1 to 5] claim 1, wherein said basic compound is selected from the group consisting of the following Formulae 5 and 6.



Formula 5

(in Formula 5, R⁷ and R⁸ represent an alkyl group or alkenyl group having 1 to 8 carbon atoms, where R⁷ and R⁸ may be the same or different. X⁴ and X⁵ represent -O- or -OCO-, where X⁴ and X⁵ may be the same or different. R⁹ and R¹⁰ represent an alkyl group or alkenyl group having 10 to 20 carbon atoms, where R⁹ and R¹⁰ may be the same or different. t is an integer of 1 to 6.)



Formula 6

Attachment to Preliminary Amendment dated December 26, 2001

Marked-up Claims

(in Formula 6, R⁷, R⁸ and R⁹ represent an alkyl group or alkenyl group having 1 to 8 carbon atoms, where R⁷, R⁸ and R⁹ may be the same or different. X4 and X5 represent -O- or -OCO-, where X4 and X5 may be the same or different. R⁹ and R¹⁰ represent an alkyl group or alkenyl group having 10 to 20 carbon atoms, where R⁹ and R¹⁰ may be the same or different. t is an integer of 1 to 6.).

7.(Amended) The liposome according to [any one of claims 1 to 6] claim 1, wherein said phosphoric acid monoester derivative is selected from predonisolone phosphate, riboflavin phosphate, and phosphatidic acid.

8.(Amended) The liposome according to [any one of claims 1 to 7] claim 1, wherein said compound having a carboxyl group or its salt, is a fatty acid.

9.(Amended) The liposome according to [any one of claims 1 to 8] claim 1, wherein said fatty acid is oleic acid, stearic acid, palmitic acid, or myristic acid.

10.(Amended) The liposome according to [any one of claims 1 to 9] claim 1, wherein the liposome membrane constituent other than [1] and [2], is phospholipid or its derivative, and/or sterol or its derivative.

Attachment to Preliminary Amendment dated December 26, 2001

Marked-up Claims

11.(Amended)The liposome according to [any one of claims 1 to 10] claim 1, wherein said drug intended for the therapy and/or diagnosis is an anti-cancer agent, an antibiotic, an enzyme agent, an enzyme inhibitor, an antioxidant, a lipid uptake inhibitor, a hormone agent, an anti-inflammatory agent, a steroid agent, a vasodilator, an angiotensin converting enzyme inhibitor, an angiotensin receptor antagonist, a growth/migration inhibitor for smooth muscle cells, a platelet aggregation inhibitor, an anticoagulant, a chemical mediator releasing inhibitor, a vascular endothelial cell growth or suppressing agent, an aldose reductase inhibitor, a mesangium cell growth inhibitor, a lipoxygenase inhibitor, an immunosuppressor, an immunoactivator, an antiviral agent, a Maillard reaction inhibitor, an amyloidosis inhibitor, an NOS inhibitor, an AGEs inhibitor, or a radical scavenger.

12.(Amended)The liposome according to [any one of claims 1 to 10] claim 1, wherein said drug intended for the therapy and/or diagnosis is a nucleic acid, a polynucleotide, a gene and its analogue.

13.(Amended)The liposome according to [any one of claims 1 to 10] claim 1, wherein said drug intended for the therapy and/or diagnosis is glycosaminoglycan and its derivative.

Attachment to Preliminary Amendment dated December 26, 2001

Marked-up Claims

14.(Amended) The liposome according to [any one of claims 1 to 10] claim 1, wherein said drug intended for the therapy and/or diagnosis is oligo- and/or polysaccharide, and derivative thereof.

15.(Amended) The liposome according to [any one of claims 1 to 10] claim 1, wherein said drug intended for the therapy and/or diagnosis is protein or peptide.

16.(Amended) The liposome according to [any one of claims 1 to 10] claim 1, wherein said drug intended for the therapy and/or diagnosis is an intracorporeal diagnostic drug such as an X-ray contrasting medium, a radiolabeled nuclear medicinal diagnostic drug, or a nuclear magnetic resonance diagnostic drug for diagnosis.

DESCRIPTION

LIPOSOME

TECHNICAL FIELD

The present invention relates to a liposome including a drug.

BACKGROUND ART

In recent years, there have been increasing studies on drug delivery system (DDS) that can perform unfailingly, efficiently and safely targeting a drug, DNA, peptide and protein to an objective site, i.e., a diseased site, for example, cancer, pneumonia, hepatitis, nephritis, lymphoma, injured site of vascular endothelia or the like.

There may be cited, for example, a method in which closed vesicles such as liposome, emulsions, lipid microspheres, and nano particles are utilized as a drug carrier, a method in which a drug is included in or bound to a polymer carrier such as high molecular synthetic polymer micelle or polysaccharide, a method in which such a closed vesicle or a polymer carrier is modified on its surface with a high molecular functional molecule such as antibody or protein, or a low molecular functional molecule

such as a specified sugar chain, peptide or the like to increase target directivity, and so forth. However, there are various problems to be overcome in practical use of these drug carriers. Among them, difficulty of evasion from the foreign matter recognizing mechanism of organism and control of pharmacokinetics in the body cause problems. In particular, closed vesicles have been difficult to perform delivery to a targeted tissue or cell with high selectivity due to its aggregation by the interaction with opsonin protein or plasma protein in blood or capture in reticuloendothelial system tissue (RES) such as liver or spleen.

As a means for solving this problem, it has been made possible to prevent adsorption of plasma protein, opsonin protein or the like to increase the stability in blood to evade capture in the RES by coating the surface of a polymer carrier inclusive of the closed vesicles with a hydrophilic polymer such as polyethylene glycol (PEG). Closed vesicles such as liposome are passively accumulated in a tissue in which the vascular permeability has been augmented in tumor tissues or at inflammatory sites because they can provide long residence in blood. This makes it possible to perform efficient treatment. On the other hand, in the case where the closed vesicles have acquired

properties of evading RES on the basis of the above-mentioned modification, the interaction between liposome and the target tissue is decreased. This improves the delivery of the closed vesicles to the target tissue. On the contrary, this causes defects in that the efficiency of incorporation of the closed vesicles by the cells in the target tissue is decreased to thereby decrease the efficiency of delivery of a drug enclosed in the closed vesicles to the cells, or the disintegration of liposome caused by the interaction with a biocomponent such as protein in blood is inhibited, with the result that the release rate of the drug enclosed in the closed vesicles is decreased.

Many liposomes comprising basic compounds as membrane components have hitherto been studied as tools for transporting genes into cells. Liposomes comprising various basic compounds in the membranes have affinity for substances having a negative charge. In particular, the cell surface is constituted by a substance having a negative charge like a sulfated polysaccharide such as heparan sulfate and hence liposomes comprising basic compounds are expected to have strong affinity for the cell membrane. However, such a liposome comprising a basic compound when it is administered in blood binds to a

substance having a negative charge such as protein in serum or the surface of erythrocyte membrane, so that the surface charge is changed or aggregation in blood is caused and loss of affinity for the cell membrane is concerned about. Therefore, a drug delivery system is desired that circulates stably having no interaction with protein or cells in blood flow and has increased interaction with cells only in a diseased site so that it can deliver the drug to the cells.

To solve these problems, various environment responsive drug carriers, that is, drug carriers that change properties in response to a change in environment due to diseases, or a difference in environment between a normal tissue and a diseased site have been studied. For example, it is known that at a diseased site such as tumor or inflammation, local pH is lowered as compared with normal tissue (V. Menkin, Biochemical Mechanism in Inflammation, Thomas, Springfield, III, pp.69-7 (1956)) and studies have been approached directed to releasing or targeting a drug in response to an environment due to a pH change at such a lesion site (Biochim Biophys Acta, 1329(2), 291-301 (1997); FEBS, 421(1), 61-4 (1998)). However, no report has been made yet on a liposome that responds to a change in pH, improving target directivity

thereof.

DISCLOSURE OF THE INVENTION

The present invention has been made to overcome the defects of conventional target directive liposome and provide a target directive liposome that responds to any environmental change. That is, an object of the present invention is to provide a liposome that exhibits target directive capability for a drug, DNA, peptide and protein by a change in pH at a diseased site.

The liposome of the present invention under such a physiological pH condition as in circulating blood weakly interacts with a substance having a negative charge, and hence it interacts with cells and proteins less frequently, whereby it is stable also in blood. However, at a target site where pH is decreased such as an inflammatory site or a tumor site, its interaction with a substance having a negative charge is enhanced, and thus its affinity for cells is improved, and it is efficiently accumulated at the target site along with that its accumulation outside the target site is prevented. As a result, an effect of alleviation of side effects can be expected.

The above-mentioned problems can be solved by the present invention as described below.

(1) The present invention relates to a liposome that includes a drug intended for the therapy and/or diagnosis, comprising as membrane constituents [1] a basic compound, [2] an acidic compound selected from a phosphoric acid monoester derivative, or a compound having a carboxyl group or its salt, and [3] a liposome membrane constituent other than [1] and [2].

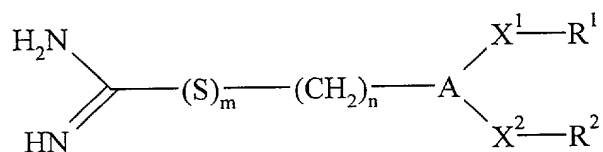
(2) The present invention relates to a liposome that includes a drug intended for the therapy and/or diagnosis, comprising as membrane constituents [1] a basic compound, [2] an acidic compound selected from a phosphoric acid monoester derivative, or a compound having a carboxyl group or its salt, and [3] other liposome membrane constituent, that is efficiently accumulated at a diseased site such as an inflammatory site or a tumor site in an environment of pH 5 to 7.

(3) The present invention relates to the liposome according to the above item (1), in which a molar ratio of the basic compound is 1 to 30 mol% of total liposome membrane constituents.

(4) The present invention relates to the liposome according to the above item (1), in which a molar ratio of the acidic compound, which is a phosphoric acid monoester derivative or a carboxylic acid derivative, is 0.5 to 30

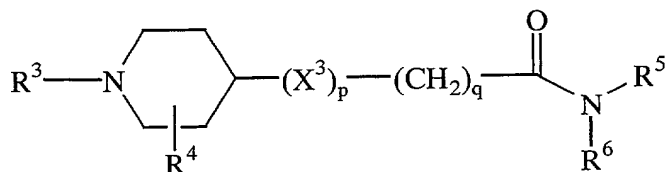
mol% of total liposome membrane constituents.

(5) The present invention relates to the liposome according to the above item (1), in which the basic compound is represented by any one of the following Formulae 1 to 4:



Formula 1

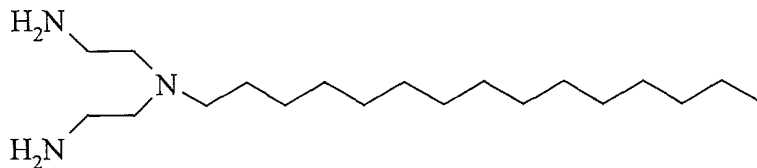
(in Formula 1, A represents an aromatic ring. R^1 and R^2 represent an alkyl group or alkenyl group having 10 to 25 carbon atoms, where R^1 and R^2 may be the same or different. X^1 and X^2 represent $-\text{O}-$, $-\text{S}-$, $-\text{COO}-$, $-\text{OCO}-$, $-\text{CONH}-$ or $\text{NHCO}-$, where X^1 and X^2 may be the same or different. m is 0 or 1, and n is 0 or an integer of 1 to 6.)



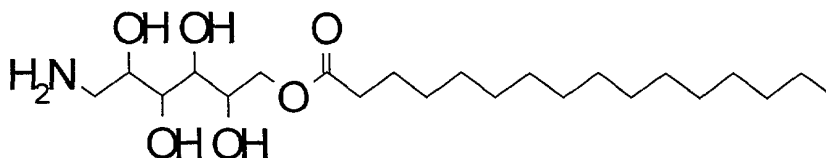
Formula 2

(in Formula 2, R^3 represents hydrogen or an alkyl group or alkenyl group having 1 to 8 carbon atoms. R^5 and R^6 represent hydrogen or an alkyl group or alkenyl group having 1 to 25 carbon atoms, (except for the case where both R^5 and R^6 are hydrogen), where R^5 and R^6 may be the

same or different. X^3 represents -O- or -S-. p is 0 or 1, and q is 0 or an integer of 1 to 10).



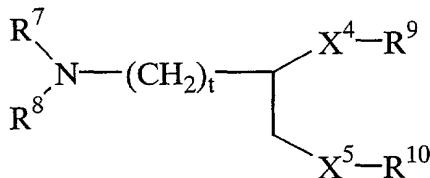
Formula 3



Formula 4

(6) The present invention relates to the liposome according to the above item (1), in which the basic compound is a basic compound having a quaternary amine or a tertiary amine.

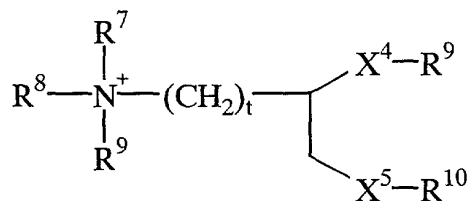
(7) The present invention relates to the liposome according to the above item (1), in which the basic compound is selected from the group consisting of the following Formulae 5 and 6.



Formula 5

(in Formula 5, R^7 and R^8 represent an alkyl group or alkenyl group having 1 to 8 carbon atoms, where R^7 and R^8 may be the same or different. X^4 and X^5 represent -O- or -

OCO-, where X^4 and X^5 may be the same or different. R^9 and R^{10} represent an alkyl group or alkenyl group having 10 to 20 carbon atoms, where R^9 and R^{10} may be the same or different. t is an integer of 1 to 6.)



Formula 6

(in Formula 6, R^7 , R^8 and R^9 represent an alkyl group or alkenyl group having 1 to 8 carbon atoms, where R^7 , R^8 and R^9 may be the same or different. X^4 and X^5 represent -O- or -OCO-, where X^4 and X^5 may be the same or different. R^9 and R^{10} represent an alkyl group or alkenyl group having 10 to 20 carbon atoms, where R^9 and R^{10} may be the same or different. t is an integer of 1 to 6.)

(8) The present invention relates to the liposome according to the above item (1), in which the phosphoric acid monoester derivative is selected from predonisolone phosphate, riboflavin phosphate, and phosphatidic acid.

(9) The present invention relates to the liposome according to the above item (1), in which the acidic compound, which is a compound having a carboxyl group or its salt, is a fatty acid.

(10) The present invention relates to the liposome

according to the above item (9), in which the fatty acid is oleic acid, stearic acid, palmitic acid, or myristic acid.

(11) The present invention relates to the liposome according to the above item (1), in which the other liposome membrane constituent is phospholipid or its derivative, and/or sterol or its derivative.

(12) The present invention relates to the liposome according to the above item (1), in which the drug intended for the therapy and/or diagnosis is an anti-cancer agent, an antibiotic, an enzyme agent, an enzyme inhibitor, an antioxidant, a lipid uptake inhibitor, a hormone agent, an anti-inflammatory agent, a steroid agent, a vasodilator, an angiotensin converting enzyme inhibitor, an angiotensin receptor antagonist, a growth/migration inhibitor for smooth muscle cells, a platelet aggregation inhibitor, an anticoagulant, a chemical mediator releasing inhibitor, a vascular endothelial cell growth or suppressing agent, an aldose reductase inhibitor, a mesangium cell growth inhibitor, a lipoxygenase inhibitor, an immunosuppressor, an immunoactivator, an antiviral agent, a Maillard reaction inhibitor, an amyloidosis inhibitor, an NOS inhibitor, an AGEs inhibitor, or a radical scavenger.

(13) The present invention relates to the liposome according to the above item (1), in which the drug intended

for the therapy and/or diagnosis is a nucleic acid, a polynucleotide, a gene and its analogue.

(14) The present invention relates to the liposome according to the above item (1), in which the drug intended for the therapy and/or diagnosis is glycosaminoglycan or its derivative.

(15) The present invention relates to the liposome according to the above item (1), in which the drug for the therapy and/or diagnosis is oligo- and/or polysaccharide, or its derivative.

(16) The present invention relates to the liposome according to the above item (1), in which the drug intended for the therapy and/or diagnosis is protein or peptide.

(17) The present invention relates to the liposome according to the above item (1), in which the drug intended for the therapy and/or diagnosis is an intracorporeal diagnostic drug such as an X-ray contrast medium, a radiolabeled nuclear medicinal diagnostic drug or a nuclear magnetic resonance diagnostic drug for diagnosis.

(18) A method of increasing a ratio of accumulation of liposome at a diseased site, comprising adding to a liposome base material including a drug for a therapy and/or diagnosis [1] a basic compound and [2] an acidic compound, which is a phosphoric acid monoester derivative

or a compound having a carboxyl group or its salt, such that a ratio of an adsorption amount of chondroitin sulfate C to liposome in a phosphate buffer of pH 6.5 to that in a phosphate buffer of pH 7.4 is at least 1.5.

To the liposome of the present invention, a lipid derivative of a hydrophilic polymer as a surface modifying agent may be added as needed, and its molecular weight is desirably 1,000 to 7,000. As the lipid derivative of the hydrophilic polymer, polyethylene glycol derivatives are desirable. In particular, compounds having a polyethylene glycol chain and diacyl glycerol in one molecule are desirable.

(19) A method for the therapy and/or diagnosis, comprising administering to an animal including a human a liposome including a drug for the therapy and/or diagnosis comprising as liposome membrane constituents [1] a basic compound, [2] an acidic compound, which is a phosphoric acid monoester derivative or a compound having a carboxyl group or its salt, and [3] liposome membrane constituent other than the above items [1] and [2] to accumulate it at a diseased site at a pH of 5 to 7.

(20) A method of using a liposome including a drug intended for the therapy and/or diagnosis comprising as liposome membrane constituents [1] a basic compound, [2] an

acidic compound, which is a phosphoric acid monoester derivative or a compound having a carboxyl group or its salt, and [3] liposome membrane constituent other than the above items [1] and [2] for accumulating the drug intended for the therapy and/or diagnosis at a diseased site at a pH of 5 to 7.

BRIEF DESCRIPTION OF DRAWINGS

Fig. 1 is a graph illustrating affinity of each liposome to proteoglycan (chondroitin sulfate C).

Fig. 2 is a graph illustrating affinity of each liposome to proteoglycan (chondroitin sulfate C).

Fig. 3 is a graph illustrating affinity of each liposome to proteoglycan (chondroitin sulfate C).

Fig. 4 is a graph illustrating affinity of each liposome to human aorta smooth muscle primary cell.

BEST MODE FOR CARRYING OUT THE INVENTION

Hereinafter, explanation will be made on embodiments of the present invention. The constituent of the liposome of the present invention is composed of a basic compound that gives to a membrane a positive charge from at near physiological pH to acidic conditions when it is embedded in the membrane by itself, an acidic compound that has a

negative charge at physiological pH but ionization of which is suppressed to decrease a negative charge at near physiological pH to acidic side when it is embedded into the membrane by itself, and another liposome constituent components. It is not particularly limited and can be used as far as it forms a microspheric structure and has safety.

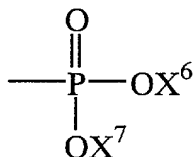
Here, the physiological pH condition means the pH in the living body, that is, around 7.4, approximately in the range of 7.2 to 7.6. The acidic side than near physiological pH indicates the acidic side than near pH 7.4, that is, the acidic side approximately at a pH in the range of 6 to 8. The acidic compounds, whose dissociation is prevented from this region, are expected to exhibit the performance due to a combination with the basic compound. However, compounds whose dissociation is prevented only in extremely acidic region such as pH 4 or less, for example, phosphoric acid diester derivatives, do not have such an action and hence are not included in the acidic compound of the present invention as will be apparent from the examples described hereinbelow.

Specific examples of the constituent of liposome membrane of the present invention include the basic compound represented by any one of the Formulae 1 to 6 above, an acidic compound, which is a phosphoric acid

monoester derivative or a compound having a carboxyl group or its salt, and another liposome membrane constituent, and it is not particularly limited and can be used as far as it forms a microspheric structure and has safety.

As the basic compound represented by any one of the Formulae 1 to 4 above, each may be properly selected and used. In particular, amidine derivatives, piperidine derivatives, primary amine derivatives, secondary amine derivatives, tertiary amine derivatives, quaternary amine derivatives, and so forth are desirable. The amount of the basic compound is preferably 1 to 30, further 5 to 30 mol% of the total liposome membrane constituent. In this range, the accumulation to a diseased site such as an inflammatory site or a tumor site at a pH of 5 to 7 is increased.

The phosphoric acid monoester derivative refers to a compound having a phosphonato group, the phosphonato group being



Formula 7

(X⁶ and X⁷ represent hydrogen or a pharmaceutically acceptable cation such as sodium or potassium, where X⁶ and X⁷ may be the same or different.)

There is no particular limitation thereon unless the

structural stability of liposome is deteriorated. Specifically, there can be mentioned phosphoesters of steroid such as prednisolone phosphate, riboflavin phosphate, and phosphatidic acid. In particular, the acidic compound, which is phosphoric acid monoester derivative is desirable as a constituent of the liposome of the present invention since it is specifically accumulated at a diseased site such as an inflammatory site or a tumor site in the region of acidic pH.

The compound having a carboxyl group or its salt is a compound having a -COOX^8 group, where X^8 represents hydrogen or a pharmaceutically acceptable cation such as sodium or potassium. A fatty acid is not particularly limited unless the structural stability of liposome is deteriorated. Specifically, there can be cited natural or synthetic oleic acid, palmitic acid, stearic acid, myristic acid, linolenic acid and so forth.

The amount of the acidic compound, which is a phosphoric monoester derivative or a carboxylic acid derivative, is preferably 0.5 to 30 mol% of the total liposome membrane constituent. In this range, accumulation at a diseased site such as an inflammatory site or a tumor site at a pH of 5 to 7 is increased.

The present invention is characterized by adding in

addition to [1] a basic compound, [2] a specified acidic compound as liposome membrane constituent.

Phosphatidylcholine that has been conventionally used as a lipid is a phosphoric acid diester compound. However, this is a dipolar ionic compound having an acidic group and a basic group in one molecule and is neutral at near physiological pH.

As the other liposome membrane constituents excluding the above-mentioned basic compound and the above-mentioned acidic compound, there can be cited phospholipids and sterols such as cholesterol. As the phospholipid, there can be cited natural or synthetic phospholipids, for example, phosphatidylcholine (lecithin), phosphatidylglycerol, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, sphingomyelin, cardiolipin and so forth, and those obtained by hydrogenating these by a common method.

The liposome membrane of the present invention may further contain a stabilizer, which includes, for example, sterol such as cholesterol, and sugars such as glycerol and sucrose that decrease membrane fluidity. As an antioxidant, there can be cited, for example, tocopherol homologues, that is, vitamin E. Tocopherol exists as four isomers α , β , γ and δ . Any one may be used in the present

invention.

A lipid derivative of a hydrophilic polymer that modifies the liposome surface is not particularly limited unless it deteriorates the structural stability of liposome. There can be cited, for example, polyethylene glycol, dextran, pullulan, ficoll, polyvinyl alcohol, synthetic polyamino acid, amylose, amylopectin, mannan, cyclodextrin, pectin, carrageenan, and derivatives thereof. Among them, polyethylene glycol and polyethylene glycol derivatives are desirable.

The molecular weight of the lipid derivative of the hydrophilic polymer is preferably 1,000 to 7,000. In particular, polyethylene glycol and polyethylene glycol derivatives having a molecular weight of 1,000 and 7,000 have considerable effect of improving retention in blood and thus are preferred. The polyethylene glycol derivative is not particularly limited. However, it is preferably a compound having a polyethylene glycol chain and a diacyl glycerol in one molecule.

To modify the liposome surface with the hydrophilic polymer, various methods may be cited and not particularly limited. However, a method in which the above-mentioned hydrophilic polymer is bound to a hydrophobic compound such as a long-chain aliphatic alcohol, sterol, a

polyoxypropylene alkyl, a glycerol fatty acid ester or a phospholipid and the part of the hydrophobic compound is inserted into the liposome surface is preferred.

As a drug for the diagnosis and/or therapy, pharmaceutically acceptable pharmacologically active substance, physiologically active substance and/or substance for diagnosis may be used depending on the purpose. The kind of drug is not particularly limited unless the formation of liposome is not damaged.

Specifically, there may be cited an anti-cancer agent, an antibiotic, an enzyme agent, an antioxidant, a lipid uptake inhibitor, a hormone agent, an anti-inflammatory agent, a steroid agent, a vasodilator, an angiotensin converting enzyme inhibitor, an angiotensin receptor antagonist, a growth/migration inhibitor for smooth muscle cells, a platelet aggregation inhibitor, an anticoagulant, a chemical mediator releasing inhibitor, a vascular endothelial cell growth accelerating or suppressing agent, an aldose reductase inhibitor, a mesangium cell growth inhibitor, a lipoxygenase inhibitor, an immunosuppressor, an immunoactivator, an antiviral agent, a Maillard reaction inhibitor, an amyloidosis inhibitor, a nitrogen monoxide synthesis inhibitor, an advanced glycation endproducts inhibitor, a radical scavenger, a protein, a peptide, a

nucleic acid, a polynucleotide, a gene and its analogues and so forth.

For example, as the examples of anti-cancer agent, there can be cited cyclophosphamide, ifosfamide, nitrogen mustard N-oxide hydrochloride, thiotepa, busulfan, carboquone, nimustine hydrochloride, ranimustine, melphalan, improsulfan tosylate, dacarbazine, procarbazine hydrochloride, cytarabine, cytarabine oxfate, enocitabine, mercaptopurine, thioinosine, fluorouracil, doxifluridine, tegafur, methotrexate, carmofur, hydroxycarbamide, vincristine sulfate, vinblastine sulfate, vindesine sulfate, etoposide, cromomycin A₃, daunorubicin hydrochloride, doxorubicin hydrochloride, aclarubicin hydrochloride, pirarubicin, epirubicin hydrochloride, dactinomycin, mitoxantrone hydrochloride, bleomycin hydrochloride, peplomycin sulfate, mitomycin C, neocarzinostatin, L-asparaginase, aceglatone mitobronitol, sodium dextran sulfate, octreotide acetate, cisplatin, carboplatin, tamoxifen citrate, medroxyprogesterone acetate, sodium estramustine phosphate, goserelin acetate, leuprorelin acetate, irinotecan hydrochloride and so forth.

As the antibiotics, there can be cited benzylpenicillin potassium, benzylpenicillin benzathine, phenoxymethylpenicillin potassium, phenethicillin

potassium, cloxacillin sodium, flucloxacillin sodium,
ampicillin, sultamicillin tosylate, bacampicillin
hydrochloride, talampicillin hydrochloride, lenampicillin,
hetacillin potassium, ciclacillin, amoxicillin,
pivmecillinam hydrochloride, aspoxicillin, carbenicillin
sodium, carindacillin sodium, sulbenicillin sodium,
ticarcillin sodium, piperacillin sodium, cephaloridine,
cephalotin sodium, cefazolin sodium, cefapirin sodium,
cefradine, cephalixin, cefatrizine propylene glycol,
cefroxadine, cefaclor, cefadroxil, cefotiam hydrochloride,
cefotiam hexetil hydrochloride, cefuroxime sodium,
cefuroxime axetil, cefamandole sodium, cefdinir, cefetamet
pivoxil hydrochloride, ceftibuten, cefmetazole sodium,
cefoxitin sodium, cefotetan sodium, cefminox sodium,
cefbuperazone sodium, cefpiramide sodium, cefsulodin
sodium, cefotaxime sodium, cefoperazone sodium, ceftizoxime
sodium, cefmenoxime hydrochloride, ceftriaxone sodium,
ceftazidime, sodium cefpimizole, cefixime, cefteram
pivoxil, cefuzonam sodium, cefpodoxim proxetil, cefodizime,
cefpirome sulfate, latamoxef sodium, flomoxef sodium,
imipenem, cilastatin sodium, aztreonam, carumonam sodium,
streptomycin sulfate, kanamycin sulfate, fradiomycin
sulfate, amikacin sulfate, gentamicin sulfate, paromomycin
sulfate, bekanamycin sulfate, ribostamycin sulfate,

dibekacin sulfate, tobramycin, sisomicin sulfate, micromomicin sulfate, astromicin sulfate, netilmicin sulfate, isepamicin sulfate, arbekacin sulfate, erythromycin, kitasamycin, acetylkitasamycin, oleandomycin phosphate, josamycin, acetylspiramycin, midecamycin, midecamycin acetate, rokitamycin, roxithromycin, clarithromycin, tetracycline hydrochloride, oxytetracycline hydrochloride, tetracycline metaphosphate, demethylchlortetracycline hydrochloride, rolitetracycline, doxycycline hydrochloride, minocycline hydrochloride, chloramphenicol, chloramphenicol succinate sodium, chloramphenicol palmitate, thiamphenicol, thiamphenicol aminoacetate hydrochloride, colistin sulfate, colistin methanesulfonate sodium, polymyxin B sulfate, bacitracin, vancomycin hydrochloride, lincomycin hydrochloride, clindamycin, spectinomycin hydrochloride, fosfomycin sodium, fosfomycin calcium, and so forth.

As examples of the enzyme agent, there can be cited chymotrypsin, crystalline trypsin, streptokinase, streptodornase, hyaluronidase, urokinase, nasaruplase, alteplase, lysozyme chloride, semialkaline protinase, serrapeptase, tisokinase, duteplase, batroxobin, pronase, promeline, and so forth.

As examples of the antioxidant, there can be cited

tocopherol, ascorbic acid, uric acid and so forth.

As examples of the anti-inflammatory agent, there can be cited choline salicylate, sasapyrine, sodium salicylate, aspirin, diflunisal, frufenamic acid, mefenamic acid, floctafenine, tolfenamic acid, diclofenac sodium, tolmetin sodium, sulindac, fenbufen, felbinac ethyl, indomethacin, indomethacin farnesil, acemetacin, proglumetacin maleate, amfenac sodium, nabumetone, ibuprofen, flurubiprofen, flurubiprofen axetil, ketoprofen, naproxen, protizinic acid, pranoprofen, fenoprofen calcium, tiaprofenic acid, oxaprozin, loxoprofen sodium, alminoprofen, zaltoprofen, phenylbutazone, clofezone, ketophenylbutazone, piroxicam, tenoxicam, ampiroxicam, tiaramide hydrochloride, tinoridine hydrochloride, benzydamine hydrochloride, epirizole, emorfazone, and so forth.

As examples of the steroid agent, there can be cited cortisone acetate, hydrocortisone (phosphoester, acetate), hydrocortisone butyrate, hydrocortisone succinate sodium, prednisolone (acetate, succinate, tertiary butylacetate ester, phosphoester), methyprednisolone (acetate), methylprednisolone succinate sodium, triamcinolone, triamcinolone acetonide (triamcinolone acetate), dexamethasone (phosphoester, acetate, sodium phosphate, sulfuric acid ester), dexamethasone palmitate,

betamethasone (phosphate, disodium salt), paramethasone acetate, fludrocortisone acetate, halopredone acetate, clobetasol propionate, halcinonide, beclomethasone propionate, betamethasone valerate, betamethasone acetate, cortisone acetate, and so forth.

As examples of the angiotensin converting enzyme inhibitor, there can be cited alacepril, imidapril hydrochloride, temocapril hydrochloride, delapril hydrochloride, benazepuril hydrochloride, captopril, cilazapril, enalapril maleate, lisinopril, and so forth.

As examples of the vasodilator, there can be cited theophylline, diprophylline, proxiphylline, aminophylline, choline theophylline, prostaglandin, prostaglandin derivatives, alprostadil alfadex, alprostadil, limaprost alfadex, papaverine, cyclandelate, cinnarizine, bencyclane fumarate, cinepazide maleate, dilazep hydrochloride, trapidil, difenidol hydrochloride, nicotinic acid, inositol hexanicotinate, nicametate citrate, nicotinic alcohol tartrate, tocopherol nicotinate, hepronicate, isoxsuprine hydrochloride, bamethan sulfate, torarizone hydrochloride, dihydroergotoxine mesylate, ifenprodil tartrate, moxislyte hydrochloride, nicergoline, nicardipine hydrochloride, nilvadipine, nifedipine, benidipine hydrochloride, diltiazem hydrochloride, nisoldipine, nitrendipine,

manidipine hydrochloride, barnidipine hydrochloride, efonidipine hydrochloride, verapamil hydrochloride, trimetazidine hydrochloride, captopril, enalapril maleate, alacepril, delapril hydrochloride, cilazapril, lisinopril, benazepuril hydrochloride, hydralazine hydrochloride, todralazine hydrochloride, budralazine, cadralazine, indapamide, carbochromen hydrochloride, efloxate, etafenone hydrochloride, oxyfedrine hydrochloride, nicorandil, amyl nitrite, isosorbide nitrate, and so forth.

As examples of the growth/migration inhibitor for smooth muscle cells, there can be cited heparin sodium, dalteparin sodium (low molecular heparin), heparin calcium, dextran sulfate, and so forth.

As examples of the platelet coagulation inhibitor, there can be cited ticlopidine hydrochloride, cilostazol, ethyl icosapentate, beraprost sodium, salpgrelate hydrochloride, batroxobin, dipyridamole, and so forth.

As examples of the anticoagulant, there can be cited sodium heparin, sodium dalteparin (low molecular heparin), calcium heparin, dextran sulfate, warfarin potassium, argatroban, and so forth.

As examples of the chemical mediator releasing inhibitor, there can be cited tranilast, ketotifen fumarate, azelastine hydrochloride, oxatomide, amlexanox,

repirinast, and so forth.

As examples of the immunosuppressor, there can be cited cyclosporine and so forth.

As examples of the antiviral agent, there can be cited aciclovir, ganciclovir, didanocine, zidovudine, sorivudine, vidarabine, and so forth.

The kind of drug for diagnosis to be included is not particularly limited unless it does not harm the formation of drug carrier. Specifically, X-ray contrasting medium, radiolabeled nuclear medicine diagnostic drug, a nuclear magnetic resonance diagnostic drug for diagnosis, and so forth.

For example, as examples of X-ray contrast medium, there can be cited amidotrizoate meglumine, iotalamate sodium, iotalamate meglumine, gastrographin, iodamide meglumine, lipiodol ultrafluid, adipiodone meglumine, ioxaglic acid, iotroxate meglumine, iotrolan, iopanoic acid, iopamidol, iohexol, ioversol, iopodate sodium, iomeprol, isopaque, iodoxamic acid, and so forth.

The liposome of the present invention can be readily obtained by a common method. One example of such a method will be described below. Constituents such as a basic compound and an acidic compound, and other constituents such as a phospholipid, a stabilizer, and antioxidant are

mixed in an organic solvent such as chloroform in a flask and the organic solvent is evaporated. Thereafter, drying under vacuum is performed to form a thin film on the inner wall of the flask. Then, a drug is added to the flask and vigorous stirring is performed, thereby obtaining a liposome dispersion. The obtained liposome dispersion is centrifuged and the supernatant is decanted to remove the drug that has not been encapsulated. Optionally, a lipid derivative solution of hydrophilic polymer may be further added as needed and the mixture is heated to obtain the liposome of the present invention.

The liposome of the present invention can be obtained also by adding a lipid derivative solution of hydrophilic polymer at the time of mixing the constituents. The method of adding the lipid derivative solution of hydrophilic polymer can be performed at any time without causing a particular problem. However, in the case of the method of adding it at the time of mixing the constituents, the lipid derivative of hydrophilic polymer is incorporated also in the inside of liposome and it may be sometimes the case that there occurs a substantial decrease in surface modification ratio and a decrease in encapsulated volume.

As an alternative method, the constituents may be mixed and the mixture may be discharged at a high pressure

by use of a high pressure discharging type emulsifier to obtain the liposome of the present invention.

The method of administering the liposome of the present invention is not particularly limited and the administration is intravenous administration as a solution or the like method. The liposome of the present invention when administered to living organisms specifically accumulates in a region of acidic pH such as an inflammatory site or a tumor site, and in other regions, its accumulation is prevented. In consideration of this, it is suitable as a vehicle for anti-inflammatory agent, anti-tumor agent or the like and has the effects of effectively causing the potency of the drugs to be exhibited and of alleviating their side effects.

Hereinafter, the present invention will be illustrated specifically along with examples. However, the present invention should not be limited thereto.

(Example 1)

Preparation of liposome comprising 3,5-dipentadecyloxybenzamidinium hydrochloride (basic compound) and myristic acid (fatty acid) as constituents

(1-[1]) First, a lipid mixed solution obtained by dissolving 4.62 mmol of phosphatidylcholine, 4.62 mmol of cholesterol, and 0.76 mmol of 3,5-

dipentadecyloxybenzamidinium hydrochloride in 100 ml of t-butyl alcohol was frozen in an ice bath and freeze-dried overnight to obtain a lipid mixture.

(1-[2]) 0.06 mmol of the lipid mixture obtained by the procedure in (1-[1]) was metered in a 50-ml eggplant type flask and 2 ml of a 1.14 mM myristic acid/chloroform solution was added thereto. After removal of the chloroform, the mixture was dried under vacuum overnight to form a lipid thin film on the inner wall of the flask.

To this, 6 ml of physiological saline was added and the mixture was subjected to ultrasonication at 55°C for 5 minutes to obtain a liposome dispersion. This was extruded under pressure by use of a polycarbonate film with a pore size of 0.4- μ m, and then by use of a polycarbonate film with the pore size of 0.2- μ m and a polycarbonate film with the pore size of 0.1- μ m. Thus the entitled liposome dispersion having an average particle size of 90 to 200 nm was obtained. The entitled liposome dispersion comprising 7.3 mol% of the basic compound [1] of the present invention and 3.7 mol% of the acidic compound [2] of the present invention was obtained. The average particle size of the obtained liposome was 125.5 nm.

(Example 2)

Preparation of liposome comprising 3,5-

dipentadecyloxybenzamidinium hydrochloride and phosphatidic acid (phosphoric acid monoester derivative) as constituents

0.06 mmol of the lipid mixture obtained by the procedure in (1-[1]) was metered in a 50-ml eggplant type flask and 0.5 ml of a 1.14 mM phosphatidic acid/chloroform solution was added thereto. After removal of the chloroform, the mixture was dried under vacuum overnight to form a lipid thin film on the inner wall of the flask. To this, 6 ml of physiological saline was added and the mixture was subjected to ultrasonication at 55°C for 5 minutes to obtain a liposome dispersion. Subsequently, the entitled liposome dispersion having an average particle size of 90 to 200 nm and comprising 7.5 mol% of the basic compound [1] of the present invention and 0.9 mol% of the acidic compound [2] of the present invention was obtained in the same manner as in (1-[2]) above. The average particle size of the obtained liposome was 130.7 nm.

(Example 3)

Preparation of liposome comprising 3,5-dipentadecyloxybenzamidinium hydrochloride (basic compound) and sodium prednisolone phosphate (phosphoric acid monoester derivative) as constituents

To 0.6 mmol of the lipid mixture obtained by the procedure in (1-[1]) was added 10 ml of a 75-mM aqueous

sodium prednisolone phosphate solution and the mixture was subjected to ultrasonication at 55°C for 5 minutes to obtain a liposome dispersion. This was subjected by particle size selection by feeding it under pressure by use of a polycarbonate film with a pore size of 0.4- μ m, and then by use of a polycarbonate film with a pore size of 0.2- μ m and a polycarbonate film with a pore size of 0.1- μ m. The obtained liposome dispersion was applied to a column (Sephacrose 4 Φ 3cm \times 20 cm, eluent: physiological saline) to collect liposome fraction to obtain the entitled liposome dispersion having a particle size of 90 to 200 nm (average particle size 130.7 nm).

(Example 4)

Preparation of liposome comprising 3,5-dipentadecyloxybenzamidinium hydrochloride (basic compound) and sodium riboflavin phosphate (phosphoric acid monoester derivative) as constituents

To 0.6 mmol of the lipid mixture obtained by the procedure in (1-[1]) was added 10 ml of a 75-mM aqueous sodium riboflavin phosphate solution and the mixture was subjected to ultrasonication at 55°C for 5 minutes to obtain a liposome dispersion. Subsequently, the same operation as in Example 3 was followed to obtain the entitled liposome dispersion. The average particle size of

the obtained liposome was 117.9 nm

(Example 5)

Preparation of liposome comprising 1,2-dipalmitoyl-3-trimethylammoniumpropane (basic compound) and myristic acid (fatty acid) as constituents

25 μ mol of phosphatidylcholine, 25 μ mol of cholesterol, 25 μ mol of 1,2-dipalmitoyl-3-trimethylammoniumpropane and 25 μ mol of myristic acid were dissolved in 10 ml of chloroform and then the chloroform was removed by use of an evaporator. Thereafter, the mixture was dried under vacuum overnight to form a lipid thin film on the inner wall of the flask.

To this, 5 ml of physiological saline was added and the mixture was subjected to ultrasonication at 55°C for 5 minutes to obtain a liposome dispersion. Subsequently, a liposome dispersion having an average particle size of 90 to 200 nm was obtained in the same manner as in (1-[2]) above. Same volumes of a 0.4-mM polyethylene glycol-phosphatidylethanolamine derivative saline solution and of the liposome dispersion were added and then the mixture was heated at 60°C for 30 minutes to obtain the entitled liposome dispersion comprising 25 mol% of the basic compound [1] of the present invention, 25 mol% of the acidic compound [2] of the present invention, and 2 mol% of

polyethylene glycol-phosphatidylethanolamine derivative.

(Example 6)

Preparation of liposome comprising 1,2-dipalmitoyl-3-trimethylammoniumpropane (basic compound) and myristic acid (fatty acid) as constituents

20 μ mol of phosphatidylcholine, 20 μ mol of cholesterol, 30 μ mol of 1,2-dipalmitoyl-3-trimethylammoniumpropane and 30 μ mol of myristic acid were dissolved in 10 ml of chloroform and then the chloroform was removed by use of an evaporator. Thereafter, the mixture was dried under vacuum overnight to form a lipid thin film on the inner wall of the flask.

To this, 5 ml of physiological saline was added and the mixture was subjected to ultrasonication at 55°C for 5 minutes to obtain a liposome dispersion. Subsequently, a liposome dispersion having an average particle size of 90 to 200 nm was obtained in the same manner as in (1-[2]) above. Same volumes of a 0.4-mM polyethylene glycol-phosphatidylethanolamine derivative saline solution and of the liposome dispersion were added and then the mixture was heated at 60°C for 30 minutes to obtain the entitled liposome dispersion comprising 30 mol% of the basic compound [1] of the present invention, 30 mol% of the acidic compound [2] of the present invention, and 2 mol% of

polyethylene glycol-phosphatidylethanolamine derivative was obtained.

Preparation was tried in the same manner as above by use of 10 μmol of phosphatidylcholine, 10 μmol of cholesterol, 40 μmol of 1,2-dipalmitoyl-3-trimethylammoniumpropane, and 40 μmol of myristic acid. However, no liposome dispersion could be obtained.
(Example 7)

Preparation of liposome comprising 1,2-dipalmitoyl-3-dimethylammoniumpropane (basic compound) and palmitic acid (fatty acid)

35 μmol of phosphatidylcholine, 35 μmol of cholesterol, 20 μmol of 1,2-dipalmitoyl-3-dimethylammoniumpropane and 10 μmol of palmitic acid were dissolved in 10 ml of chloroform and then the chloroform was removed by use of an evaporator. Thereafter, the mixture was dried under vacuum overnight to form a lipid thin film on the inner wall of the flask.

To this, 5 ml of physiological saline was added and the mixture was subjected to ultrasonication at 55°C for 5 minutes to obtain a liposome dispersion. Subsequently, a liposome dispersion having an average particle size of 90 to 200 nm was obtained in the same manner as in (1-[2])

above. Same volumes of a 0.4-mM polyethylene glycol-phosphatidylethanolamine derivative saline solution and of the liposome dispersion were added and then the mixture was heated at 60°C for 30 minutes to obtain the entitled liposome dispersion comprising 20 mol% of the basic compound [1] of the present invention, 10 mol% of the acidic compound [2] of the present invention, and 2 mol% of polyethylene glycol-phosphatidylethanolamine derivative was obtained.

(Example 8)

Preparation of liposome comprising 1,2-dipalmitoyl-3-dimethylammoniumpropane (basic compound) and palmitic acid (fatty acid) as constituents

30 μ mol of phosphatidylcholine, 30 μ mol of cholesterol, 20 μ mol of 1,2-dipalmitoyl-3-dimethylammoniumpropane and 20 μ mol of palmitic acid were dissolved in 10 ml of chloroform and then the chloroform was removed by use of an evaporator. Thereafter, the mixture was dried under vacuum overnight to form a lipid thin film on the inner wall of the flask.

To this, 5 ml of physiological saline was added and the mixture was subjected to ultrasonication at 55°C for 5 minutes to obtain a liposome dispersion. Subsequently, a liposome dispersion having an average particle size of 90

to 200 nm was obtained in the same manner as in (1-[2]) above. Same volumes of a 0.4-mM polyethylene glycol-phosphatidylethanolamine derivative saline solution and of the liposome dispersion were added and then the mixture was heated at 60°C for 30 minutes to obtain the entitled liposome dispersion comprising 20 mol% of the basic compound [1] of the present invention, 20 mol% of the acidic compound [2] of the present invention, and 2 mol% of polyethylene glycol-phosphatidylethanolamine derivative was obtained.

(Example 9)

Preparation of liposome comprising 3,5-dipentadecyloxybenzamidinium hydrochloride (basic compound) and phosphatidic acid as constituents

44 μmol of phosphatidylcholine, 44 μmol of cholesterol, 10 μmol of 3,5-dipentadecyloxybenzamidinium hydrochloride and 2 μmol of phosphatidic acid were dissolved in 10 ml of chloroform and then the chloroform was removed by use of an evaporator. Thereafter, the mixture was dried under vacuum overnight to form a lipid thin film on the inner wall of the flask.

To this, 5 ml of physiological saline was added and the mixture was subjected to ultrasonication at 55°C for 5 minutes to obtain a liposome dispersion. Subsequently, a

liposome dispersion having an average particle size of 90 to 200 nm was obtained in the same manner as in (1-[2]) above. Same volumes of a 0.12-mM polyethylene glycol-phosphatidylethanolamine derivative saline solution and of the liposome dispersion were added and then the mixture was heated at 60°C for 30 minutes to obtain the entitled liposome dispersion comprising 10 mol% of the basic compound [1] of the present invention, 2 mol% of the acidic compound [2] of the present invention, and 0.6 mol% of polyethylene glycol-phosphatidylethanolamine derivative was obtained.

(Comparative Example 1)

To 0.6 mmol of the lipid mixture obtained by the procedure above-mentioned in (1-[1]) was added 10 ml of physiological saline and the mixture was subjected to ultrasonication at 55°C for 5 minutes to obtain a liposome dispersion. This was processed in the same manner as in (1-[2]) above to obtain the entitled liposome dispersion having an average particle size of 90 to 200 nm. The average particle size of the obtained liposome was 110.5 nm.

(Comparative Example 2)

Preparation of liposome comprising 3,5-dipentadecyloxybenzamidinium hydrochloride and

phosphatidylglycerol as constituents

0.06 mmol of the lipid mixture obtained by the procedure above-mentioned in (1-[1]) was metered in a 50-ml eggplant type flask and 2 ml of a 1.14 mM phosphatidylglycerol/chloroform solution was added thereto. After removal of the chloroform, the mixture was dried under vacuum overnight to form a lipid thin film on the inner wall of the flask. To this, 6 ml of physiological saline was added and the mixture was subjected to ultrasonication at 55°C for 5 minutes to obtain a liposome dispersion. This was processed in the same manner as in (1-[2]) above to obtain the entitled liposome dispersion having an average particle size of 90 to 200 nm. The average particle size of the obtained liposome was 138.8 nm.

(Comparative Example 3)

Preparation of liposome comprising 3,5-dipentadecyloxybenzamidinium hydrochloride and polyethylene glycol-phosphatidylethanolamine derivative as constituents

The lipid concentration of the liposome dispersion obtained in Comparative Example 1 was adjusted to 20 mM. To this liposome dispersion was added same volume of polyethylene glycol- phosphatidylethanolamine derivative solution (molecular weight : about 6,000, and concentration

: 1.214 mg/ml) and then the mixture was heated at 60°C for 30 minutes to obtain the entitled liposome dispersion having a particle size of 90 to 200 nm (average particle size : 117.4nm).

(Comparative Example 4)

Preparation of liposome comprising 1,2-dipalmitoyl-3-trimethylammoniumpropane (basic compound) as a constituent

37.5 μ mol of phosphatidylcholine, 37.5 μ mol of cholesterol, and 25 μ mol of 1,2-dipalmitoyl-3-trimethylammoniumpropane were dissolved in 10 ml of chloroform and then the chloroform was removed by use of an evaporator. Thereafter, the mixture was dried under vacuum overnight to form a lipid thin film on the inner wall of the flask.

To this, 5 ml of physiological saline was added and the mixture was subjected to ultrasonication at 55°C for 5 minutes to obtain a liposome dispersion. Subsequently, a liposome dispersion having an average particle size of 90 to 200 nm was obtained in the same manner as in (1-[2]) above. Same volumes of a 0.4-mM polyethylene glycol-phosphatidylethanolamine derivative saline solution and of the liposome dispersion were added and then the mixture was heated at 60°C for 30 minutes to obtain the entitled liposome dispersion.

(Comparative Example 5)

Preparation of liposome comprising 1,2-dipalmitoyl-3-trimethylammoniumpropane (basic compound) as a constituent

35 μmol of phosphatidylcholine, 35 μmol of cholesterol, and 30 μmol of 1,2-dipalmitoyl-3-trimethylammoniumpropane were dissolved in 10 ml of chloroform and then the chloroform was removed by use of an evaporator. Thereafter, the mixture was dried under vacuum overnight to form a lipid thin film on the inner wall of the flask.

To this, 5 ml of physiological saline was added and the mixture was subjected to ultrasonication at 55°C for 5 minutes to obtain a liposome dispersion. Subsequently, a liposome dispersion having an average particle size of 90 to 200 nm was obtained in the same manner as in (1-[2]) above. Same volumes of a 0.4-mM polyethylene glycol-phosphatidylethanolamine derivative saline solution and of the liposome dispersion were added and then the mixture was heated at 60°C for 30 minutes to obtain the entitled liposome dispersion.

(Comparative Example 6)

50 μmol of phosphatidylcholine, and 50 μmol of cholesterol were dissolved in 10 ml of chloroform and then the chloroform was removed by use of an evaporator.

Thereafter, the mixture was dried under vacuum overnight to form a lipid film on the inner wall of the flask.

To this, 5 ml of physiological saline was added and the mixture was subjected to ultrasonication at 55°C for 5 minutes to obtain a liposome dispersion. Subsequently, a liposome dispersion having an average particle size of 90 to 200 nm was obtained in the same manner as in (1-[2]) above. Same volumes of a 0.12-mM polyethylene glycol-phosphatidylethanolamine derivative saline solution and of the liposome dispersion were added and then heated at 60°C for 30 minutes to obtain the liposome dispersion not comprising the basic compound [1] of the present invention and the acidic compound [2] of the present invention.
(Comparative Example 7)

45 μ mol of phosphatidylcholine, 45 μ mol of cholesterol, and 10 μ mol of 3,5-dipentadecyloxybenzamidinium hydrochloride were dissolved in 10 ml of chloroform and then the chloroform was removed by use of an evaporator. Thereafter, the mixture was dried under vacuum overnight to form a lipid thin film on the inner wall of the flask.

To this, 5 ml of physiological saline was added and the mixture was subjected to ultrasonication at 55°C for 5 minutes to obtain a liposome dispersion. Subsequently, a liposome dispersion having an average particle size of 90

to 200 nm was obtained in the same manner as in (1-[2]) above. Same volumes of a 0.12-mM polyethylene glycol-phosphatidylethanolamine derivative saline solution and of the liposome dispersion were added and then heated at 60°C for 30 minutes to obtain the liposome dispersion.

(Comparative Example 8)

49 μ mol of phosphatidylcholine, 49 μ mol of cholesterol, and 2 μ mol of phosphatidic acid were dissolved in 10 ml of chloroform and then the chloroform was removed by use of an evaporator. Thereafter, the mixture was dried under vacuum overnight to form a lipid thin film on the inner wall of the flask.

To this, 5 ml of physiological saline was added and the mixture was subjected to ultrasonication at 55°C for 5 minutes to obtain a liposome dispersion. Subsequently, a liposome dispersion having an average particle size of 90 to 200 nm was obtained in the same manner as in (1-[2]) above. Same volumes of a 0.12-mM polyethylene glycol-phosphatidylethanolamine derivative saline solution and of the liposome dispersion were added and then heated at 60°C for 30 minutes to obtain the liposome dispersion comprising an anionic lipid.

(Test Example 1) Affinity of various kinds of liposomes for

proteoglycan

The purpose of this test was to examine the affinity of liposome for proteoglycan overproduced at an inflammatory site or a tumor site under acidic condition and in the vicinity of neutrality.

(Method)

Glycosaminoglycan serving as an anionic sugar chain polymer, contained in a proteoglycan was mixed with liposome in each of Examples and Comparative Examples and the amount of glycosaminoglycan adsorbed on each liposome was measured. Chondroitin sulfate C (molecular weight : 30,000 to 60,000) as the glycosaminoglycan was dissolved in a phosphate buffer at pH 7.4 and adjusted to 0.1 mg/ml and further adjusted to pH 6.0 or pH 6.5 with 6N HCl. 0.3 ml of the obtained 0.1 mg/ml chondroitin sulfate C solution at pH 7.4 or pH 6.0 and 0.3 ml of each liposome dispersion of which the lipid concentration was adjusted to 10 mM with physiological saline were mixed and heated at 37°C for 1 hour. After cooling, ultracentrifugation was performed at 100,000 g for 1 hour and the supernatant was collected. Chondroitin sulfate C in the supernatant was determined by use of a Blyscan proteoglycan assay kit and the amount of adsorption of chondroitin sulfate C to each liposome was calculated.

(Results)

The results are shown in Figs. 1 to 3. The liposomes in Comparative Example 1 in which no acidic compound was comprised and Comparative Examples 2 to 5 and 7 in which the acidic compound was a phosphodiester had the same adsorption amount of chondroitin sulfate C at pH 6.0 or 6.5, and pH 7.4. The liposomes in Comparative Examples 6 and 8 in which no basic compound was comprised did not adsorb any chondroitin sulfate C at pH 6.5 and pH 7.4. It was confirmed that the liposomes in accordance with Examples 1 to 9 of the present invention showed some difference in the adsorption amount for chondroitin sulfate C between pH 6.0 or 6.5 and pH 7.4 and at pH 7.4, the adsorption amount was suppressed. That is, it was observed that the liposomes of the present invention had suppressed affinity for proteoglycan in the vicinity of neutrality while in acidic environment it showed high affinity therefor.

(Test Example 2) Evaluation of affinity for cells of various kinds of liposomes

(Method)

Human aorta smooth muscle primary culture cell (produced by Clonetics) was spread on 12-well culture plate

(Falcon, produced by Becton Dickinson) to 3.8×10^4 cells/well and incubated for 24 hours. The medium was changed to RPMI1640 (produced by Sigma)/10% FBS (pH 6.0 or pH 7.4) (1 ml/well), and the liposomes of Example 9 and Comparative Examples 6 to 8 were added respectively in amounts of 50 μ g PC/well and allowed to incubate for 24 hours (n=3). After washing with PBS(-) twice, the cells were lysed with a cytolytic agent (50 mM Tris (pH 8.0)/150 mM, NaCl/0.5% DOC/1% NP-40/0.1% SDS) and the fluorescence intensity was measured. PC concentration was converted from the fluorescence intensity and corrected with the amount of protein. Then the binding amount of each liposome to cell was calculated.

(Results)

The results are shown in Fig. 4. The liposome of Comparative Example 7 had approximately the same binding amount to cell at pH 6.0 and at pH 7.4. The liposomes of Comparative Examples 6 and 8 did not bind to the cells at pH 6.0 or at pH 7.4. It was confirmed that the liposome in accordance with Example 9 of the present invention showed a difference in the binding amount to the cells between pH 6.0 or 6.5 and pH 7.4 and had a suppressed binding amount at pH 7.4. That is, it was observed that the liposomes of the present invention had suppressed affinity for

proteoglycan in the vicinity of neutrality while in acidic environment it showed high affinity therefor.

(Test Example 3) Acute Toxicity

The purpose of this test was to know the degree of toxicity of the liposome of the present invention as compared with the conventional ones. For this purpose, lethal toxicity tests of the liposome of the present invention on rats were performed.

(Method)

The liposome dispersions of Examples 1 to 9 were concentrated by use of an ultrafiltration membrane and optionally diluted with sterilized distilled water for injection as needed to make test solutions. Quarantined 5-week aged mice were divided into groups of 5 animals per group and 100 ml/kg of the test solution was administered to mice via the tail vein. On the other hand, the solvent control group was administered with 100 ml/kg of physiological saline. After the administration of the test solution, the general condition was carefully observed at least once a day for 7 days and indication of toxicity and death case were recorded. Also, 7 days after administration, autopsy was performed to remove respective organs. Pathological sections were prepared for each organ

and observation was performed thereon.

(Results)

No death case was observed during the observation period in the case of the liposomes of the present invention. In the histopathological observation of each organ after 7 days, no histopathological change was observed. That is, it was confirmed that the liposomes of the present invention were extremely low in toxicity and high in safety.

INDUSTRIAL APPLICABILITY

The liposomes of the present invention are very high in targeting capability at a diseased site such as inflammation and tumor where pH has been decreased in acidic regions. Also, they are very high in safety.

Therefore, the liposomes of the present invention that carry a drug for the diagnosis and/or therapy are very effective for the purpose of the diagnosis and therapy of tissues and/or organs where inflammation or tumor lesion has occurred.

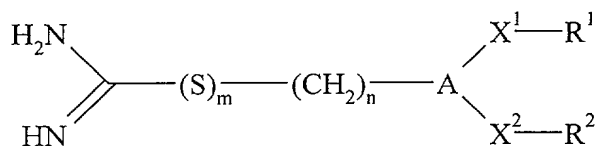
Claims

1. A liposome that includes a drug intended for the therapy and/or diagnosis, comprising as membrane constituents [1] a basic compound, [2] an acidic compound which is a phosphoric acid monoester derivative, or a compound having a carboxyl group or its salt, and [3] a liposome membrane constituent other than [1] and [2], that is accumulated at a diseased site at pH 5 to 7.

2. The liposome according to claim 1, wherein a molar ratio of said basic compound is 1 to 30 mol% of total liposome membrane constituents.

3. The liposome according to claim 1 or 2, wherein a molar ratio of said acidic compound is 0.5 to 30 mol% of total liposome membrane constituents.

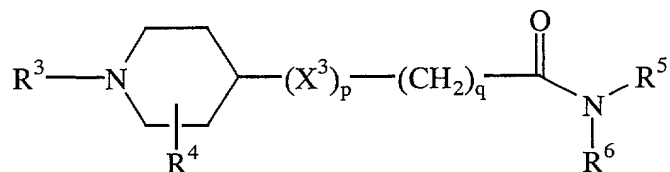
4. The liposome according to any one of claims 1 to 3, wherein said basic compound is represented by any one of the following Formulae 1 to 4:



Formula 1

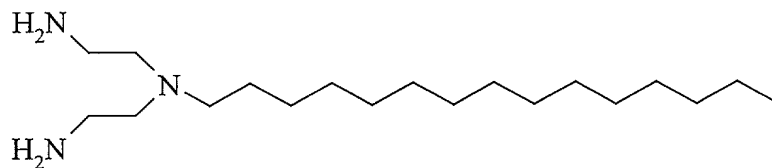
(in Formula 1, A represents an aromatic ring. R^1 and R^2

represent an alkyl group or alkenyl group having 10 to 25 carbon atoms, where R^1 and R^2 may be the same or different. X^1 and X^2 represent $-O-$, $-S-$, $-COO-$, $-OCO-$, $-CONH-$ or $NHCO-$, where X^1 and X^2 may be the same or different. m is 0 or 1, and n is 0 or an integer of 1 to 6.)

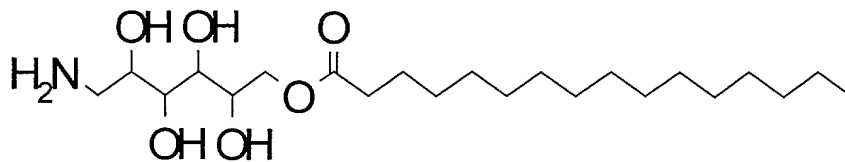


Formula 2

(in Formula 2, R^3 represents hydrogen or an alkyl group or alkenyl group having 1 to 8 carbon atoms. R^5 and R^6 represent hydrogen, or an alkyl group or alkenyl group having 1 to 25 carbon atoms, (except for the case where both R^5 and R^6 are hydrogen atoms), where R^5 and R^6 may be the same or different. X^3 represents $-O-$ or $-S-$. p is 0 or 1, and q is 0 or an integer of 1 to 10).



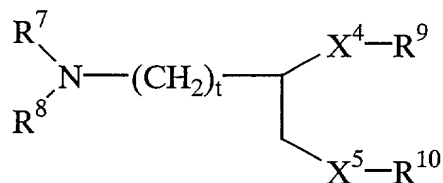
Formula 3



Formula 4

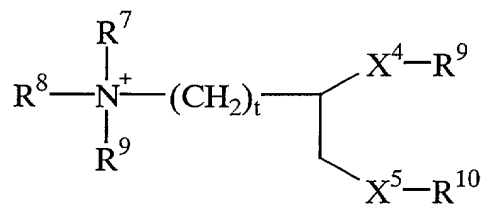
5. The liposome according to any one of claims 1 to 4, wherein said basic compound is a basic compound having a quaternary amine or a tertiary amine.

6. The liposome according to any one of claims 1 to 5, wherein said basic compound is selected from the group consisting of the following Formulae 5 and 6.



Formula 5

(in Formula 5, R^7 and R^8 represent an alkyl group or alkenyl group having 1 to 8 carbon atoms, where R^7 and R^8 may be the same or different. X^4 and X^5 represent $-\text{O}-$ or $-\text{OCO}-$, where X^4 and X^5 may be the same or different. R^9 and R^{10} represent an alkyl group or alkenyl group having 10 to 20 carbon atoms, where R^9 and R^{10} may be the same or different. t is an integer of 1 to 6.)



Formula 6

(in Formula 6, R^7 , R^8 and R^9 represent an alkyl group or

alkenyl group having 1 to 8 carbon atoms, where R^7 , R^8 and R^9 may be the same or different. X^4 and X^5 represent -O- or -OCO-, where X^4 and X^5 may be the same or different. R^9 and R^{10} represent an alkyl group or alkenyl group having 10 to 20 carbon atoms, where R^9 and R^{10} may be the same or different. t is an integer of 1 to 6.).

7. The liposome according to any one of claims 1 to 6, wherein said phosphoric acid monoester derivative is selected from predonisolone phosphate, riboflavin phosphate, and phosphatidic acid.

8. The liposome according to any one of claims 1 to 7, wherein said compound having a carboxyl group or its salt, is a fatty acid.

9. The liposome according to any one of claims 1 to 8, wherein said fatty acid is oleic acid, stearic acid, palmitic acid, or myristic acid.

10. The liposome according to any one of claims 1 to 9, wherein the liposome membrane constituent other than [1] and [2], is phospholipid or its derivative, and/or sterol or its derivative.

11. The liposome according to any one of claims 1 to 10, wherein said drug intended for the therapy and/or diagnosis is an anti-cancer agent, an antibiotic, an enzyme agent, an enzyme inhibitor, an antioxidant, a lipid uptake inhibitor, a hormone agent, an anti-inflammatory agent, a steroid agent, a vasodilator, an angiotensin converting enzyme inhibitor, an angiotensin receptor antagonist, a growth/migration inhibitor for smooth muscle cells, a platelet aggregation inhibitor, an anticoagulant, a chemical mediator releasing inhibitor, a vascular endothelial cell growth or suppressing agent, an aldose reductase inhibitor, a mesangium cell growth inhibitor, a lipoxygenase inhibitor, an immunosuppressor, an immunoactivator, an antiviral agent, a Maillard reaction inhibitor, an amyloidosis inhibitor, an NOS inhibitor, an AGEs inhibitor, or a radical scavenger.

12. The liposome according to any one of claims 1 to 10, wherein said drug intended for the therapy and/or diagnosis is a nucleic acid, a polynucleotide, a gene and its analogue.

13. The liposome according to any one of claims 1 to 10,

wherein said drug intended for the therapy and/or diagnosis is glycosaminoglycan and its derivative.

14. The liposome according to any one of claims 1 to 10, wherein said drug intended for the therapy and/or diagnosis is oligo- and/or polysaccharide, and derivative thereof.

15. The liposome according to any one of claims 1 to 10, wherein said drug intended for the therapy and/or diagnosis is protein or peptide.

16. The liposome according to any one of claims 1 to 10, wherein said drug intended for the therapy and/or diagnosis is an intracorporeal diagnostic drug such as an X-ray contrasting medium, a radiolabeled nuclear medicinal diagnostic drug, or a nuclear magnetic resonance diagnostic drug for diagnosis.

17. A method of increasing a ratio of accumulation of liposome at a diseased site, comprising adding to a liposome membrane constituent including a drug for a therapy and/or diagnosis [1] a basic compound and [2] an acidic compound, which is a phosphoric acid monoester derivative, or a compound having a carboxyl group or its

salt, such that a ratio of an adsorption amount of chondroitin sulfate C to liposome in a phosphate buffer of pH 6.5 to that in a phosphate buffer of pH 7.4 is at least 1.5.

18. A method for the therapy and/or diagnosis, comprising administering to an animal including a human a liposome including a drug intended for the therapy and/or diagnosis comprising as liposome membrane constituents [1] a basic compound, [2] an acidic compound, which is a phosphoric acid monoester derivative, or a compound having a carboxyl group or its salt, and [3] a liposome membrane constituent other than [1] and [2] to accumulate it at a diseased site at a pH of 5 to 7.

19. A method of using a liposome including a drug intended for the therapy and/or diagnosis comprising as liposome membrane constituents [1] a basic compound, [2] an acidic compound, which is a phosphoric acid monoester derivative, or a compound having a carboxyl group or its salt, and [3] a liposome membrane constituent other than [1] and [2] for accumulating the drug intended for the therapy and/or diagnosis at a diseased site at a pH of 5 to 7.

Declaration and Power of Attorney for Patent Application

特許出願宣言書及び委任状

Japanese Language Declaration

日本語宣言書

下記の氏名の発明者として、私は以下の通り宣言します。

As a below named inventor, I hereby declare that:

私の住所、私書箱、国籍は下記の私の氏名の後に記載された通りです。

My residence, post office address and citizenship are as stated next to my name.

下記の名称の発明に関して請求範囲に記載され、特許出願している発明内容について、私が最初かつ唯一の発明者（下記の氏名が一つの場合）もしくは最初かつ共同発明者であると（下記の名称が複数の場合）信じています。

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

LIPOSOME

上記発明の明細書（下記の欄でx印がついていない場合は、本書に添付）は、

the specification of which is attached hereto unless the following box is checked:

☐ 月 日に提出され、米国出願番号または特許協定条約国際出願番号を _____ とし、
(該当する場合) _____ に訂正されました。

☐ was filed on June 26, 2000
as United States Application Number or PCT
International Application Number
PCT/JP00/04182 (Confirmation
No. _____)
and was amended on _____ (if applicable)

私は、連邦規則法典第37編第1条56項に定義されるとおり、特許資格の有無について重要な情報を開示する義務があることを認めます。

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

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FIG. 1

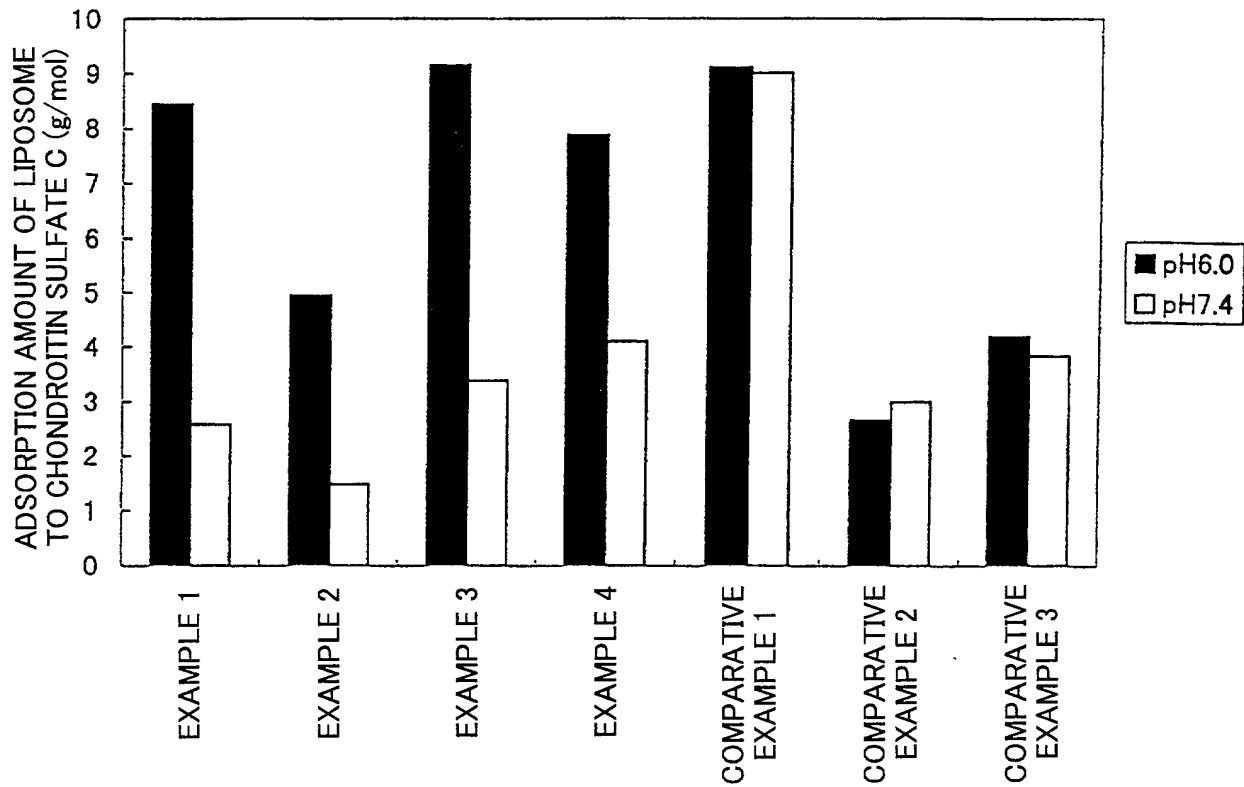
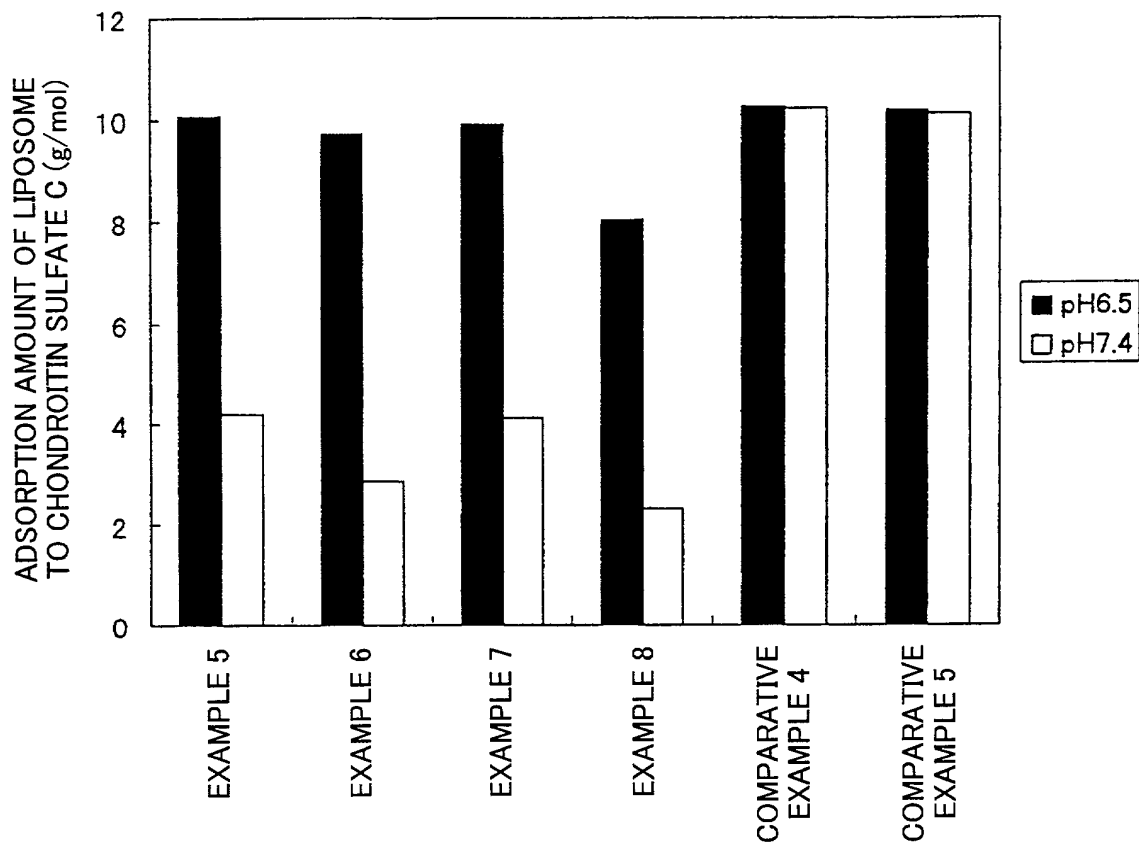


FIG. 2



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FIG. 3

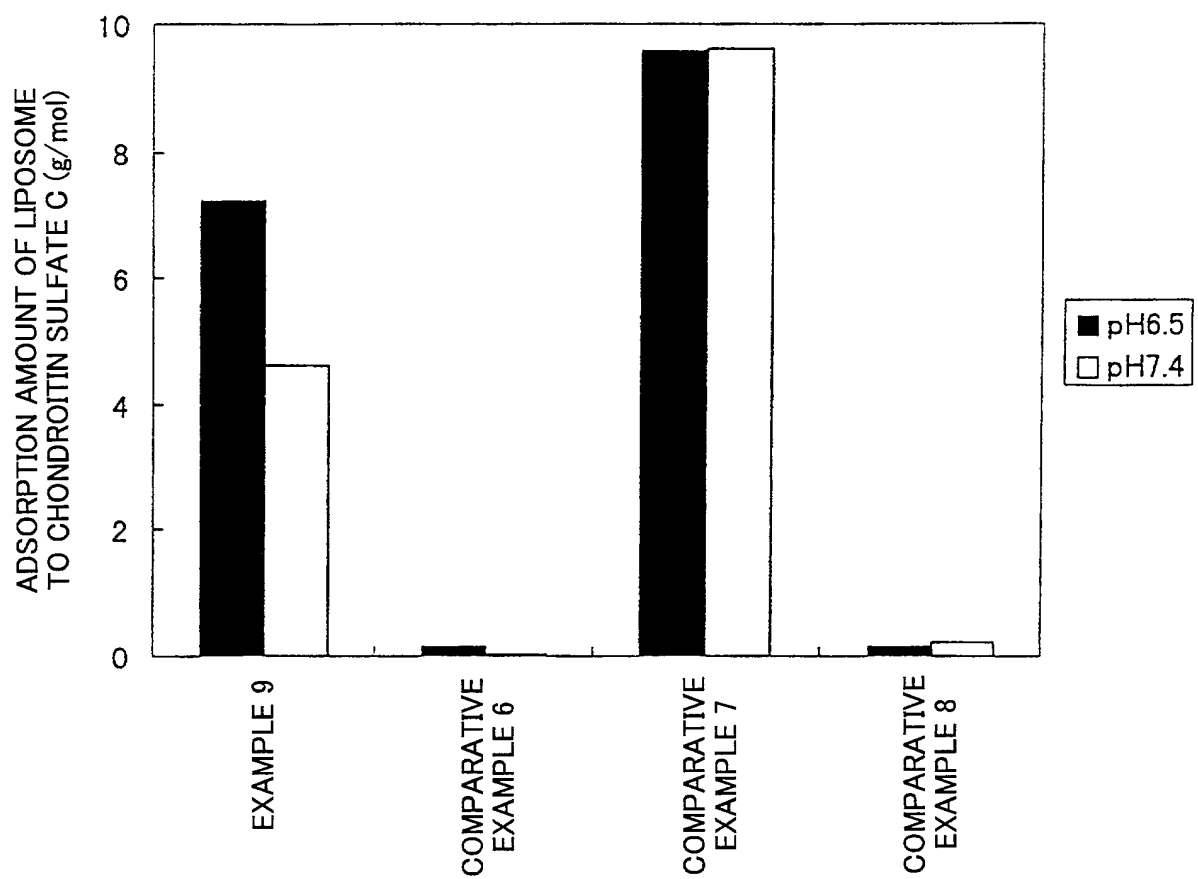
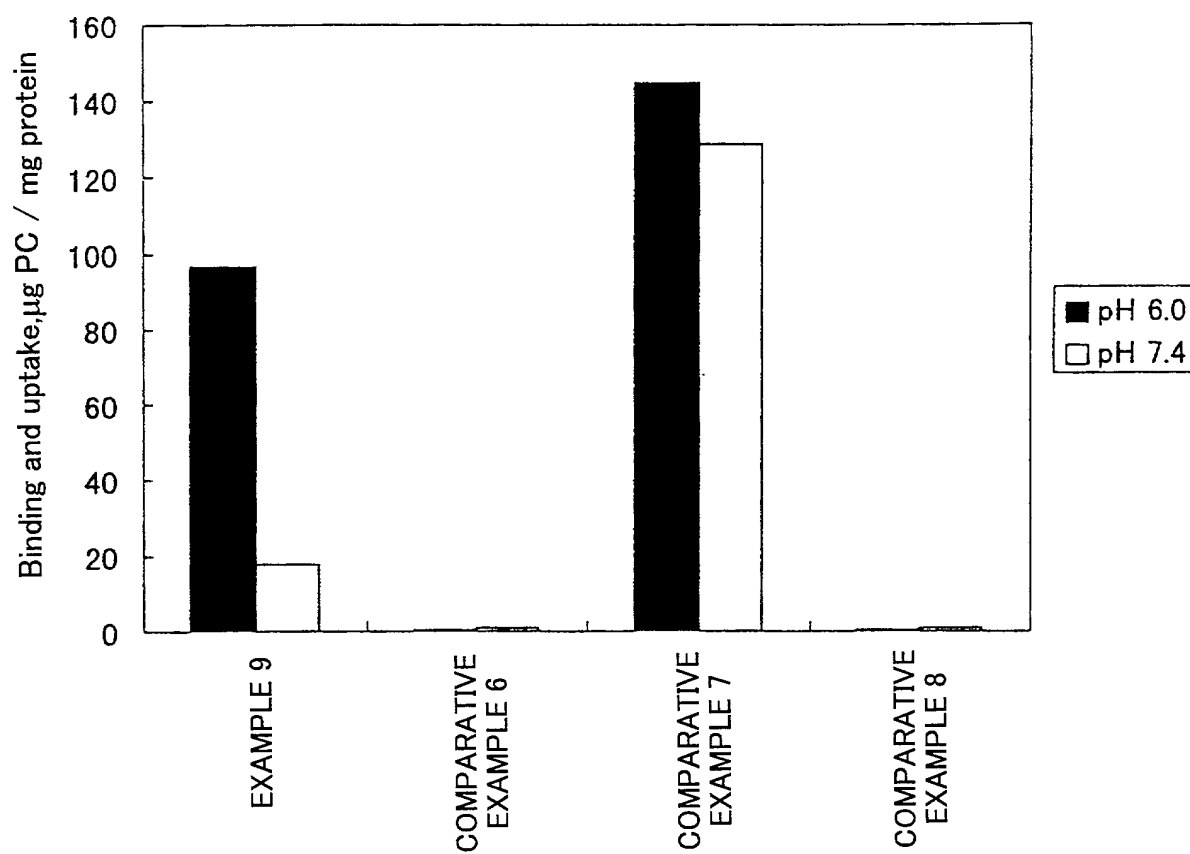


FIG. 4



Japanese Language Declaration

日本語宣言書

私は、米国法典第35編119条(a)-(d)項又は365条(b)項に基づき下記の、米国以外の国の少なくとも一カ国を指定している特許協力条約365(a)項に基づき国際出願、又は外国での特許出願もしくは発明者証の出願についての外国優先権をここに主張するとともに、優先権を主張している、本出願の前に出願された特許または発明者証の外国出願を以下に、枠内をマークすることで、示しています。

Prior foreign application(s)
外国での先行出願

Priority Not Claimed
優先権主張なし

11-180158 (Number) (番号)	Japan (Country) (国名)
 (Number) (番号)	 (Country) (国名)
 (Number) (番号)	 (Country) (国名)

I hereby claim foreign priority under Title 35, United States Code, § 119(a)-(d) or § 365 (b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

June 25, 1999 (Day/Month/Year Filed) (出願年月日)	<input type="checkbox"/>
 (Day/Month/Year Filed) (出願年月日)	<input type="checkbox"/>
 (Day/Month/Year Filed) (出願年月日)	<input type="checkbox"/>

私と、第35編米国法典119条(e)項に基づいて下記の米国特許出願規定に記載された権利をここに主張いたします。

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

(Application No.) (出願番号)	(Filing Date) (出願日)
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(Application No.) (出願番号)	(Filing Date) (出願日)
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私は、下記の米国法典第35編120条に基づいて下記の米国特許出願に記載された権利、又は米国を指定している特許協力条約365条(c)に基づき権利をここに主張します。また、本出願の各請求範囲の内容が米国法典第35編112条第1項又は特許協力条約で規定された方法で先行する米国特許出願に開示されていない限り、その先行米国出願書提出日以降で本出願書の日本国内または特許協力条約国際提出日までの期間中に入手された、連邦規則法典第37編1条56項で定義された特許資格の有無に関する重要な情報について開示義務があることを認識しています。

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

(Application No.) (出願番号)	(Filing Date) (出願日)
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(Status)(patented, pending, abandoned) (現況：特許許可済、係属中、放棄済)
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(Application No.) (出願番号)	(Filing Date) (出願日)
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(Status)(patented, pending, abandoned) (現況：特許許可済、係属中、放棄済)
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私は、私自身の知識に基づいて本宣言書で私が行なう表明が真実であり、かつ私の入手した情報と私の信じることに基づき表明が全て真実であると信じていること、さらに故意になされた虚偽の表明及びそれと同等の行為は米国法典第18編第1001条に基づき、罰金または拘禁、もしくはその両方により処罰されること、そしてそのような故意による虚偽の声明を行なえば、出願した、又は既に許可された特許の有効性が失われることを認識し、よってここに上記のごとく宣誓を致します。

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Japanese Language Declaration

日本語宣言書

委任状: 私は下記の発明者として、本出願に関する一切の手続きを米特許商標局に対して遂行する弁理士または代理人として、下記の者を指名いたします。(弁理士、または代理人の氏名及び登録番号を明記のこと)

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(Supply similar information and signature for third and subsequent joint inventors.)

Japanese Language Declaration

日本語宣言書

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第六の共同発明者の氏名 (該当する場合)			
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